An investigation of arabinan and cellulose based nano-composite fabrication methods

A Senior Project

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Bachelor of Science

by

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Approval Page

Project Title: An investigation of arabinan and cellulose based nano-composite fabrication methods

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CAL POLY STATE UNIVERSITY
Materials Engineering Department

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We would like to thank our advisor, Dr. Trevor Harding, for all of his insight, guidance, and funding during this project. Without his help, we could not have accomplished anything. We also want to thank Dr. Phil Costanzo from the Chemistry department; his input was invaluable.

Abstract/Executive Summary

Microcrystalline cellulose was converted into nanocrystalline cellulose via an acid hydrolysis procedure. Scanning electron microscopy (SEM) was employed to measure the particle size and thus the effectiveness of acid hydrolysis. The nanocrystalline cellulose was poured through a 0.2μm filter to isolate the particles of ideal size. The nanocrystalline cellulose samples were stored in dimethylformamide (DMF) to prevent mold growth and agglomeration upon drying. Numerous composite samples were created by dissolving the arabinan in a solvent, suspending cellulose in the arabinan solution, and then drying the sample. Casting was performed in a silicon mold to allow sample removal without damage. Initial casting was performed without cellulose to determine the most appropriate solvent. DMF dissolved arabinan most effectively and created the most uniform sample, so it was used almost exclusively for fabrication of the remaining samples. The subsequent arabinan and cellulose (ArC) composites were fabricated with various processing parameters such as weight percent, stir time, dry time, and dry temperature. Based on qualitative examination, the most effective casting process consisted of a 24-hour stir followed by a 55 °C vacuum drying condition. A glass transition temperature of 96 °C for arabinan was determined using differential scanning calorimetry (DSC).

Key Words: materials engineering, arabinan, cellulose, composite, materials, acid hydrolysis, microcrystalline, nanocrystalline, cactus, fabrication, spine, nano, biomimicry, microcrystalline cellulose, nanocrystalline cellulose,

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1. Introduction

1.1 Motivation

Cactus spines are a naturally occurring arabinan-cellulose composite. They have stiffness comparable to spruce wood but show remarkably high bending strength.[1] Additionally, all-cellulose composites (ACCs) have been successfully produced, and show promise for numerous uses; they have demonstrated excellent mechanical and thermal properties, are made from renewable materials, and are both recyclable and biodegradable. However, ACCs display strong hydrophilic properties and they absorb water. This makes them problematic in applications that might encounter moisture. Research suggests that incorporating arabinan, another polysaccharide found in pectins in some plants, into a cellulose composite could improve hydrophobicity. The arabinan should form a covalent bond with the cellulose and tie up some of the hydroxyl groups that make cellulose so hydrophilic.[2] Research regarding the extraction and refining of the raw materials, arabinan and cellulose, is ongoing, and some research teams have been able to successfully produce composites using cellulose and arabinoxylan, a polysaccharide similar to arabinan.

1.2 Project Goals

The goal of this project is to investigate methods for producing an arabinan and cellulose based composite that mimics the strength of cactus spines and improves on the hydrophobicity of ACCs. Commercially available microcrystalline cellulose (MCC) will be converted into nanocrystalline cellulose (NCC) fibers with a maximum diameter of 6nm. The NCC will then be dispersed in an arabinan solution and the solution will be dried.

1.3 Scope

This project is a preliminary investigation and proof of concept for ArC composites meant to provide a solid base for future work. However, due to time and resource constraints, only one fabrication method was explored thoroughly. As such, the conclusions and discussion are limited, but the procedures are detailed and the recommended future work is extensive.

1.4 Realistic Constraints

The project was constrained by economic and manufacturability factors. Arabinan is only available for purchase from a single industrial supplier, and the price of 20 USD per gram limits the production of samples. Further, the composite fabrication method should be repeatable and scalable to produce any number of samples.

2. Background

Opuntia ficus-indica (OFI) cactus spines were used as the representative model for the biomimicry aspect of this project. These spines consist of a 50/50 arabinan and cellulose fiber reinforced composite structure in which the fibers run parallel to the major spine axis.

2.1 Arabinan

Pectins are heteropolysaccharides, a polysaccharide with more than one monosaccharide present, that are often found in the cell walls of plants.[3] Arabinan is a general term referring to various polysaccharides such as arabinose that are generally found as lateral side chains on pectins. The arabinan constituent of OFI spines is 94.3% arabinose, 0.6% galactose, 1.6% rhamnose, 1.4% galacturonic acid, and 0.7% glucose.[4]

The arabinan used in this study was purchased from Megazyme International Ireland and is derived from sugar beets. The polysaccharide composition is 88% arabinose, 3% galactose, 2% rhamnose, and 7% galacturonic acid. The molecular weight is 15,000 daltons. The arabinan is readily soluble in cold water and soluble in hot water up to 20% w/v.[5]

2.2 Cellulose

Cellulose is a glucose polysaccharide, one of the most abundant biopolymers on earth, and both renewable and biodegradable (Figure 1). Further, its specific stiffness, 67 GPa cm^3/g, is among the highest of all natural materials,[2] and it has a modulus of 167.5 GPa laterally along the fibril chain.[6] SEM and TEM of OFI spine cross sections have revealed the cellulose fibers to have a lateral dimension of 6-10 μm,[7] however it should be noted that these fibers consist of smaller microfibrils with diameters no larger than 4-6 nm (Figure 2).[4] The large surface area per volume of the cellulose suggests a close, nanoscale association between the arabinan and cellulose.

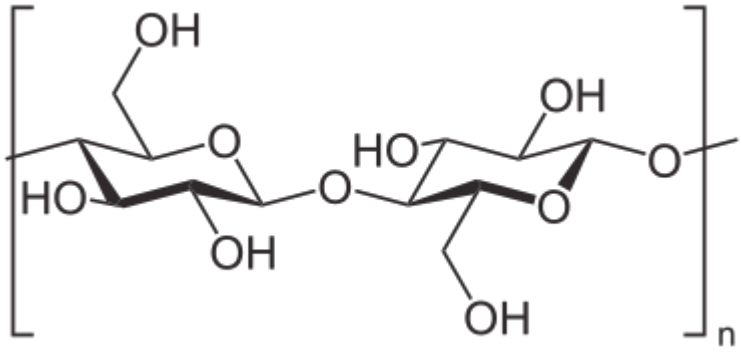

Figure 1: A single monosaccharide of glucose in cellulose showing the -OH or hydroxyl groups.[8]

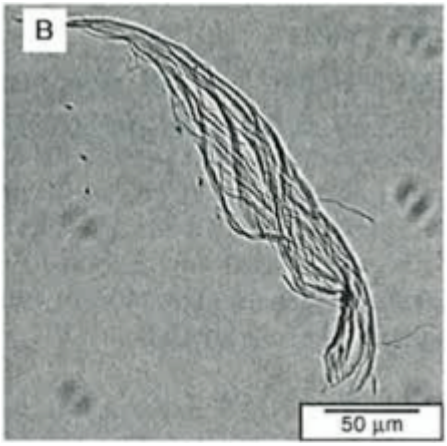

Figure 2: An example of a cellulose microfibril after alkali, bleaching, and autoclave treatment.[4]

2.3 NCC and Acid Hydrolysis

NCC is a monocrystalline cellulose whisker ten to hundreds of nanometers long, 1-100 nm in diameter, and with an aspect ratio close to 70. It has been used as a reinforcing filler to dramatically increase the strength of thermosets and thermoplastics.[9] Acid hydrolysis has proven to be an effective method of converting MCC into NCC. In this procedure, the amorphous regions are removed, particle size is reduced, and a highly crystalline rod-like NCC remains.[6] In this project, the MCC was converted to NCC to increase the surface area to volume ratio and achieve greater bonding characteristics between the arabinan and cellulose.

2.4 All-Cellulose and Arabinan-Cellulose Composites

All-Cellulose (CC) composites have been successfully produced, and show promise for numerous uses; such materials have demonstrated excellent mechanical and thermal properties, are made from renewable materials, and are biodegradable. Since they are made of one substance, they are easily recyclable. The entire composite can be reprocessed without separating the matrix from the reinforcement. Unfortunately, CC composites display strong hydrophilic properties, which make them problematic in applications that might encounter moisture. The individual cellulose molecules contain hydroxyl groups that form hydrogen bonds with water (Figure 1). In fact, the hydrophilic nature of cellulose makes it chemically incompatible with hydrophobic polymer matrices, but chemically treating the surface of the cellulose or using nanoscale cellulose particles can mitigate this issue. Research suggests that incorporating arabinan, a polysaccharide found in some plants, into ACCs could improve hydrophobicity by tying some of the hydroxyl groups into covalent bonds.[2] Research regarding the extraction and refining of the raw materials is ongoing. Some research teams have been able to successfully produce composites using cellulose and arabinoxylan, a polysaccharide similar to arabinan.

3. Procedures/SOPs

3.1 Acid Hydrolysis

See Appendix A for SOP

3.2 Filtration

See Appendix B for SOP
A filtration procedure was utilized to isolate NCC particles smaller than 2µm.

3.3 Casting

See Appendix C for SOP

3.3.1 Dissolving the arabinan

Preliminary castings of arabinan-only samples with tetrahydrofuran (THF), acetone, ethanol, methanol, and dimethylformamide (DMF) were conducted in order to determine which solvent best dissolved the arabinan. The first batch was 25 weight percent arabinan, but many of the solvents either incompletely dissolved the arabinan or did not dissolve it at all. To determine whether the solutions were too concentrated or the solvents were indeed poor matches, the next batch was prepared with 5 weight percent. For both trials, solutions with each solvent were prepared, hand stirred for 5 minutes, and allowed to dry at room temperature and pressure in a silicon mold.

3.3.2 Dispersing the cellulose

Even dispersion of the cellulose within the composite samples is critical to ensure uniform appearance and properties. To resolve cellulose clumping found in early composite samples that were hand stirred for 5 minutes, later arabinan-cellulose solutions were mechanically stirred for 24 hours in a sealed reaction vessel.

3.3.3 Drying the samples

Samples were dried either at room temperature and pressure or at 54° C in a vacuum oven. The drying temperature was chosen to not exceed the glass transition temperature (96 °C) of arabinan as found via DSC.

This fabrication method allows for various size batches of samples to be prepared at once.

4. Results

4.1 MCC-NCC

Acid hydrolysis was successful at reducing the average particle size from microcrystalline scale (average particle size of 50 µm) to nanocrystalline scale, but the particles are not uniform. An average particle size cannot be calculated via SEM. However, it can be noted that after acid hydrolysis the maximum particle diameter was reduced from well over 100um to ~25 µm (Figure 3). Further, most of the post hydrolysis particles were significantly smaller than the maximum. The cellulose used for our samples was significantly more particle shaped than the microfibrils found in OFI (Figure 2).

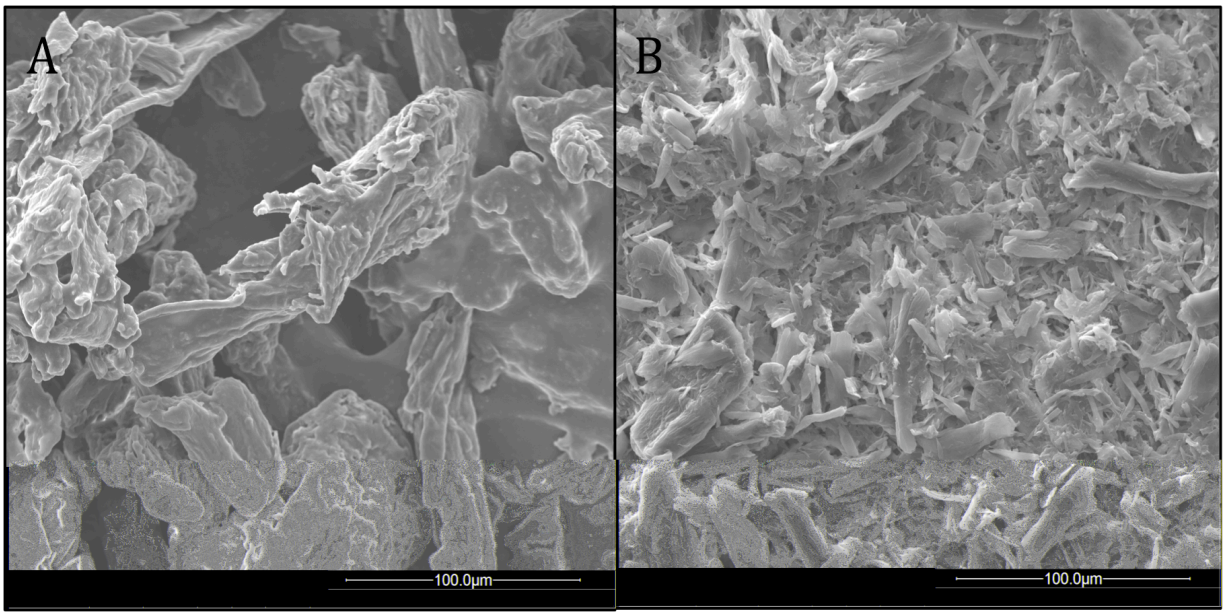

Figure 3: SEM images at 1000x magnification of cellulose (A) before and (B) after acid hydrolysis.

4.2 Casting

4.2.1 Dissolving the arabinan

For the 25 weight percent trial, THF and acetone did not dissolve the arabinan, leaving a dry powder (Figure 4). Methanol created an extremely dry and brittle sample with a cracked and uneven surface and was thus eliminated from further consideration as a solvent. Ethanol created a rough and porous solid and only partially dissolved the arabinan. DMF created a translucent and uniform sample, indicating that the arabinan was completely dissolved within the solvent.

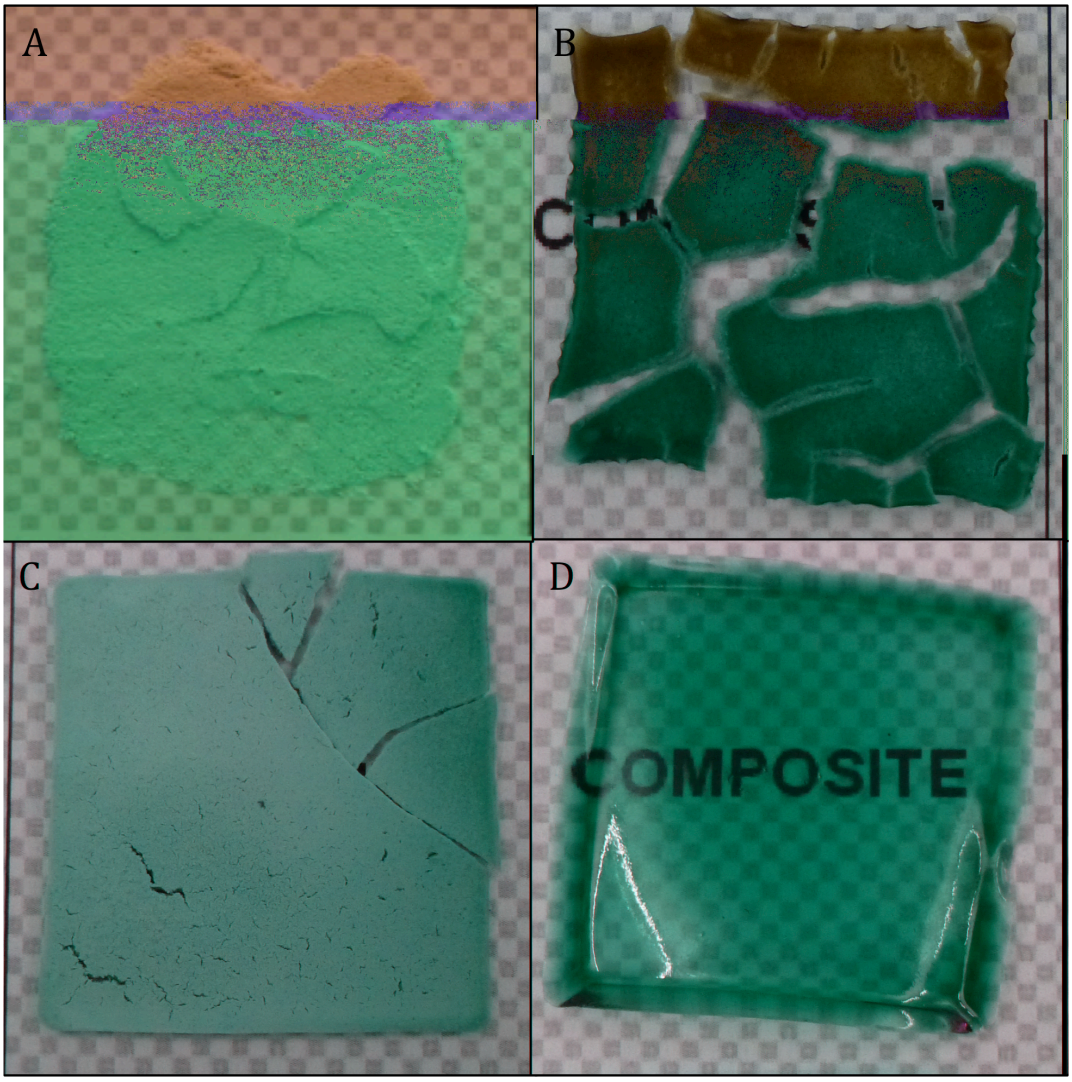

Figure 4: Arabinan at 25 wt-% in (a) THF or acetone (dry powder), (b) methanol, (c) ethanol, and (d) DMF.

The amount of arabinan was reduced to 5 weight percent to more completely explore some of the solvents (Figure 5). For this trial, THF only partially dissolved the arabinan, leaving a porous and brittle solid with a rough surface. Both ethanol and acetone produced uneven samples with incomplete dissolution. DMF was not tested during this trial because it performed well previously. Of the preliminary casting samples, DMF proved to be the most successful and was therefore used as the solvent for further casting.

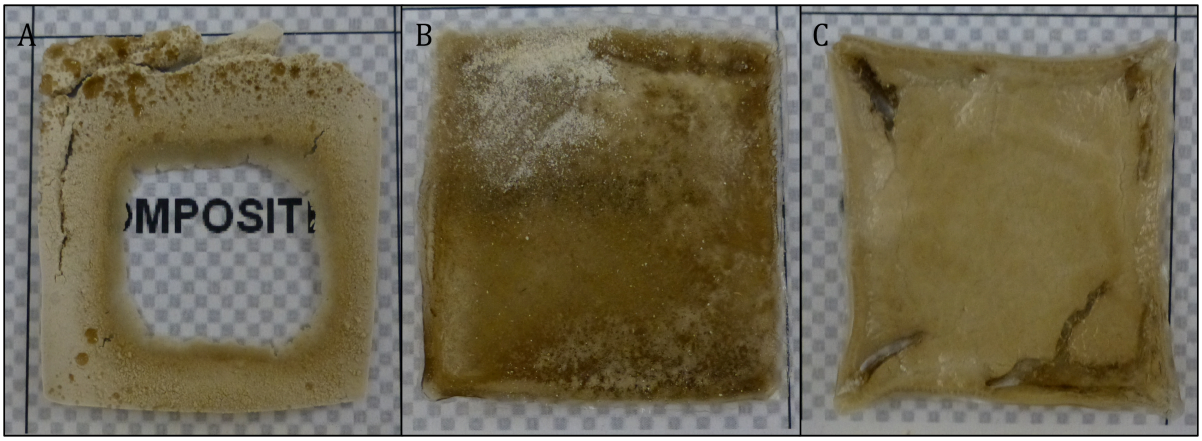

Figure 5: Samples made with 5 wt-% arabinan in (a) THF, (b) acetone, and (c) ethanol.

4.2.2 Dispersing the cellulose

The initial five-minute manual stir left clumps of cellulose within the arabinan solution (Figure 6 A). Therefore, a 24-hour mechanical stir was used to more effectively disperse the cellulose and create a uniform solution.

4.2.3 Drying the samples

Samples dried at room temperature and ambient pressure had an uneven and warped surface (Figure 6). Drying the samples in a vacuum oven at 54 °C yielded a more level surface with a matte texture. Therefore, samples were dried in a heated vacuum oven for remaining castings.

Filtered NCC was used more recently with the 24-hour stir and heated vacuum dry to create samples, but unexplained bubbling occurred while drying. A sample was then produced under the same conditions, but at ambient pressure, which yielded a smooth and uniform sample.

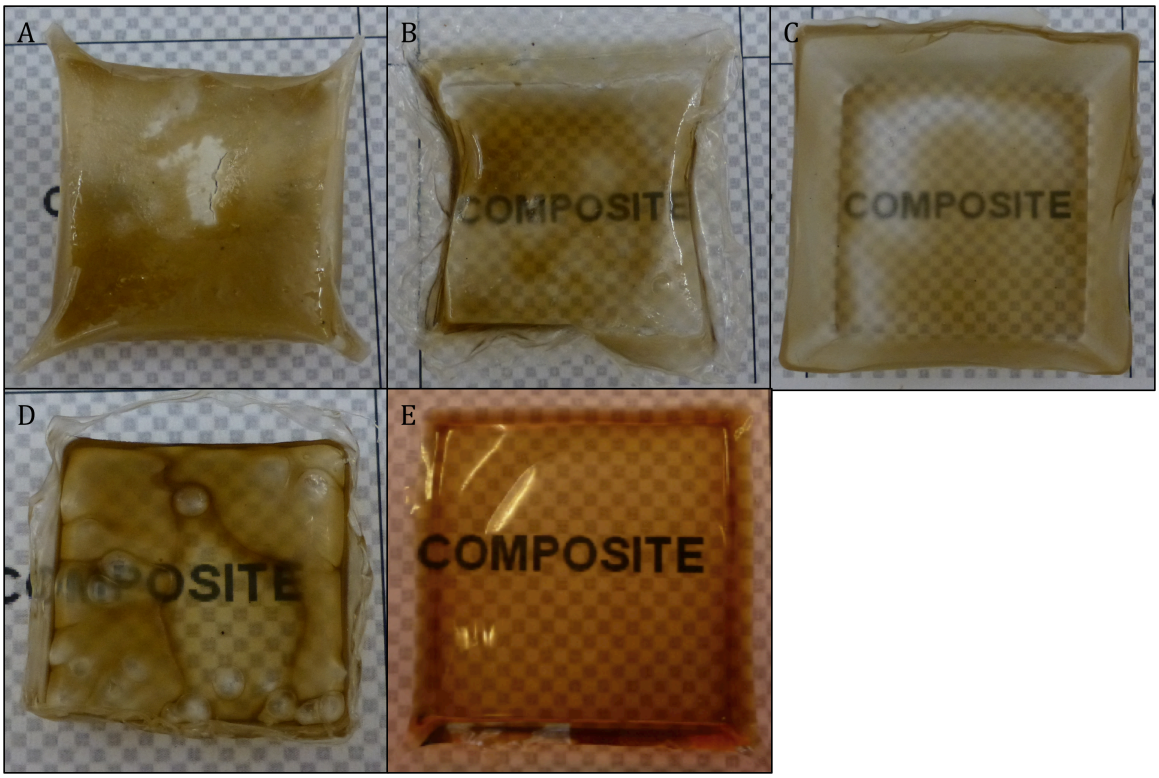

Figure 6: ArC composite samples with various processing parameters. See Table I for details.

Table I. Processing conditions for each ArC sample produced

Sample	Stir Time (min)	Dry Temperature (°C)	Dry Pressure (torr)	NCC
A	5	22	760	Unfiltered
B	1440	22	760	Unfiltered
C	1440	54	~1	Unfiltered
D	1440	54	~1	Filtered
E	1440	54	760	Filtered

5. Conclusions

The following conclusions were drawn based on the work completed thus far:

- It is possible to create an arabinan-cellulose composite
- DMF was the most effective solvent for dissolving arabinan
- A 24-hour stir time and heated vacuum dry produced the best samples based on qualitative examination.

The successes thus far suggest that arabinan and cellulose based composites are a promising development in the biopolymer field. It is important to note that final processing parameters and materials used during the entire material life cycle will determine how sustainable these composites are.

6. Suggestions for future studies

Cal Poly's SEM was not resolved enough to view the filtered NCC; therefore, the average particle size after the filtration process still needs to be determined. The use of an AFM or TEM could more effectively characterize the filtered NCC.

Both the ArC and arabinan-only samples should be tested using a low load flexure test. Quantitative comparisons of strength and stiffness can then be made amongst samples as well as with natural cactus spines to determine the optimum casting parameters.

Other casting parameters should be explored, such as the use of co-solvents, varied stir and dry conditions, and sample thickness. Post-casting heat treatments at temperatures 20-25 °C above the glass transition temperature of arabinan should be carried out. This could potentially increase the bonding and cross-linking between arabinan and NCC, which may increase the strength and stiffness of the composite.

Fracture surfaces of composite samples should be viewed using SEM to determine the distribution of NCC within each sample. XRD should also be performed on the samples made with filtered NCC to ensure the presence of cellulose, which will show as a crystalline peak within the amorphous arabinan matrix, in each sample.

For this project the fabrication method explored in most detail was casting; however, melt processing is another promising means of creating ArC composites. DSC of arabinan powder revealed a glass transition temperature at 96 °C (Figure 7), and thus melt processing can be performed at 120 °C. A method to dry the NCC, which is currently held in solution, while still preventing particle agglomeration would need to be developed prior to performing melt processing.

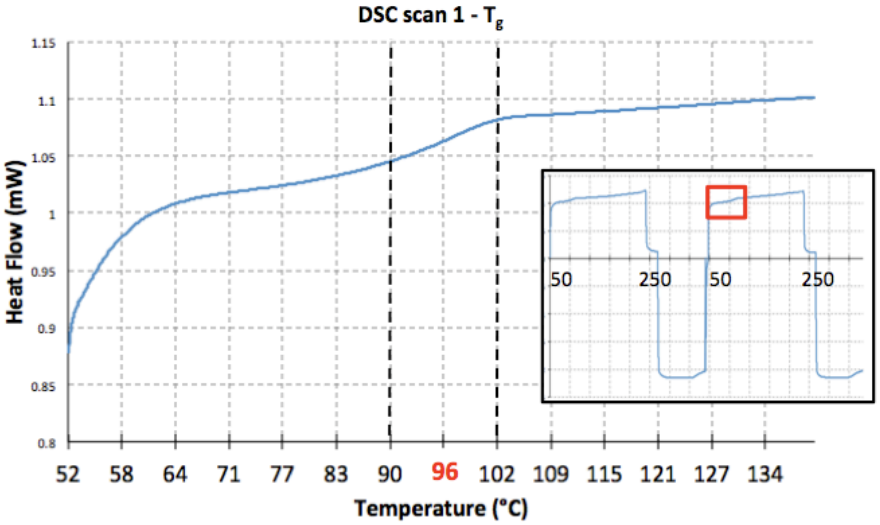

Figure 7: A DSC scan that consisted of two cycles from 50 to 250 °C were performed on arabinan. The upward shift in heat flow indicates an increase in heat capacity, revealing a glass transition temperature of approximately 96 °C.

Lastly, because arabinan is an expensive resource, development of an in-house extraction method would increase the amount of production and testing that could be done and lower the overall cost of the project.

7. Appendices

Appendix A: Acid Hydrolysis and Solvent Exchange Procedure

Standard Operating Procedure

Acid Hydrolysis and Solvent Exchange Procedure

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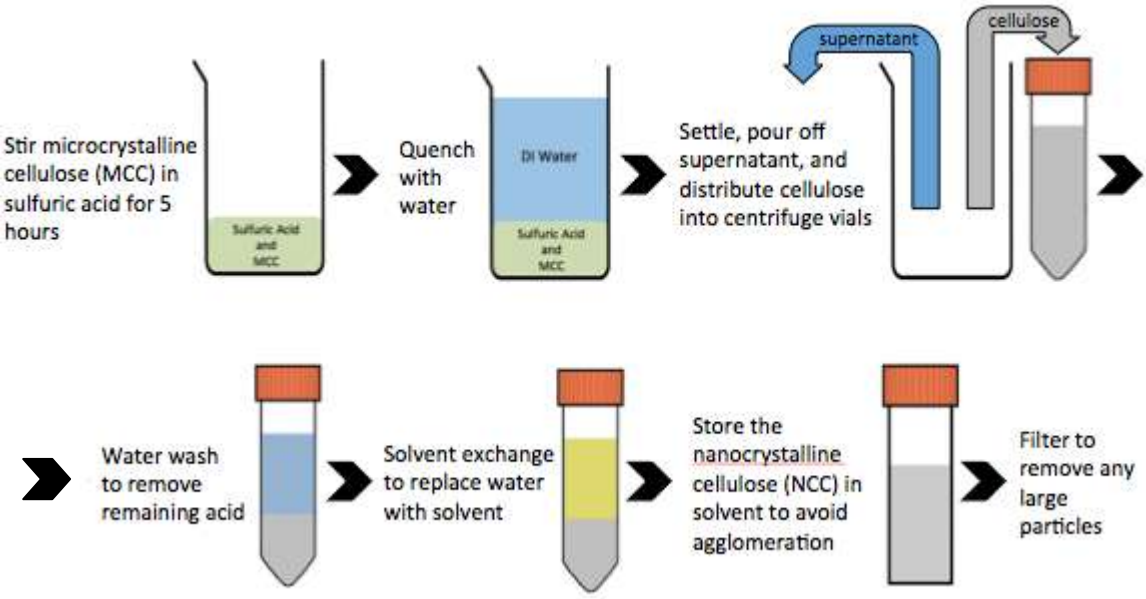
Acid Hydrolysis and Solvent Exchange Overview



Acid Hydrolysis Set Up

Note: Acid hydrolysis was originally performed in the Chemistry Department's lab, but the Material Engineering Department's labs can accommodate this procedure as of May 2013. The acid hydrolysis was performed in Bldg 192 Room 212. The solvent exchange procedure was performed in the Nanotech Lab in Bldg 41 Room 205.

DISCLAIMER: Some materials and equipment are batch size specific. This SOP was written for a batch size of 10g of cellulose.

Hot Plate/Oil Bath

1. Using a digital hot plate with stirring capabilities, heat an oil bath at 45 °C in a fume hood.	

Microcrystalline Cellulose

2. Put empty weigh-boat on scale. Close all doors to scale. “Zero” the scale.	

3. Scoop out microcrystalline cellulose (MCC) onto weigh-boat until the scale reads 10.00g with the doors of the scale closed.	

4. Carefully transfer MCC from weigh-boat into reaction vessel.	

Water and Acid

5. Measure out 56.90 mL of deionized (DI) water.	

6. Add DI water to 500 mL beaker.	

7. Place the beaker with water in it on a digital scale. “Zero” the scale. Add 56.00 g of 95-98% sulfuric acid to the water in the beaker. (CAUTION: use proper personal protection equipment (PPE): acid-resistant gloves, goggles, apron. Use proper technique while handling sulfuric acid). Always add acid to water. Never add water to acid.

8. Carefully add acid-water solution and stir bar into the reaction vessel containing the MCC.	

9. Cap the reaction vessel and insert a needle into the top of the cap in order to allow for ventilation. Secure the reaction vessel by clamping the neck of the reaction vessel. Lower the reaction vessel into the oil bath so that the solution is submerged in the oil bath, but not touching the bottom of the oil bath container. Tighten the nuts and clamps so that the reaction vessel cannot move.	

10. Allow the reaction to run for 5 hours. Note the start time on the procedure log.

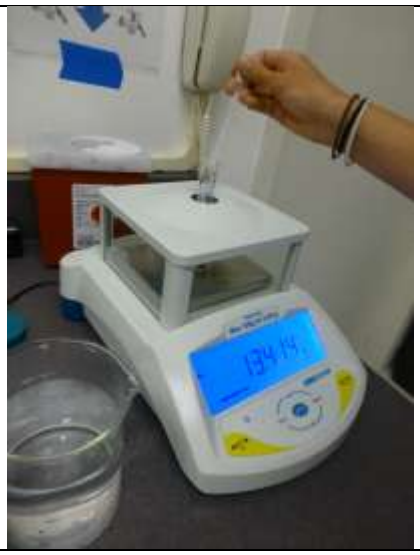
Acid Hydrolysis Procedure

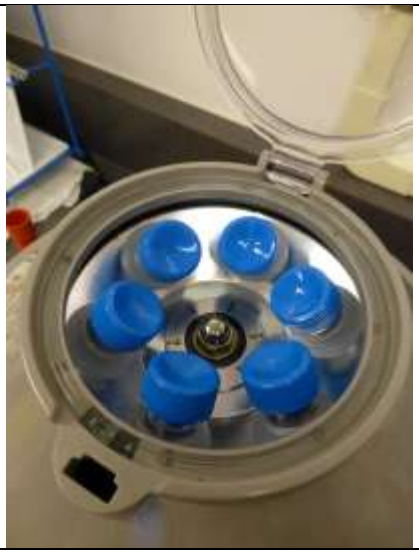
11. Remove the reaction vessel from the oil bath and clamps. Remove the needle from the top of the cap. Remove the cap from the reaction vessel. Pour the reacted solution into a 1000mL beaker. Add enough DI water to the reaction vessel to swirl around and pour into the same beaker. Add 500mL DI water to the reacted solution in order to quench the solution.	

12. Allow quenched solution to rest and separate. Most of the cellulose should fall to the bottom within 10 minutes. Once settled, pour off about 300mL of the liquid from the top layer into a 500mL beaker, which will be used as the waste beaker. NOTE: Do not pour down the sink. Acid Waste and Mixed Solvent Waste Containers can be found under the fume hood in the metallography room in Bldg 192.

Water Wash

13. Pour cellulose solution into 15 mL centrifuge vials. (Any remaining solution will be used for subsequent washes).	

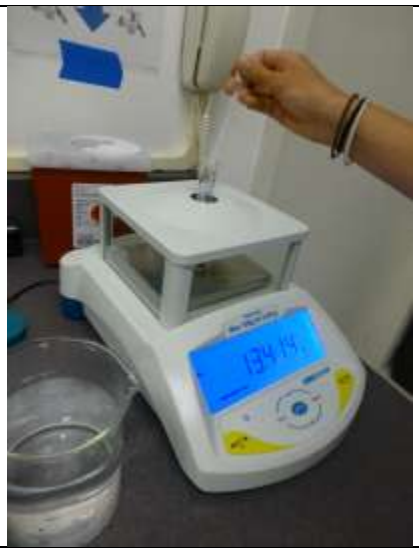
14. Using a digital scale, ensure that the vials weigh equally.	

15. Place filled and capped vials into the centrifuge slots. Make sure that the vials are distributed evenly to achieve balanced weight. Close the lid.	

16. Run the centrifuge for 2 minutes at 2500 RPM. Adjust the time and RPM using the (+) and (-) buttons if needed.	

17. Pour off liquid ("supernatant") from vials into waste beaker. A "puck" of cellulose should remain at the bottom of the vial. NOTE: A glass or wooden rod should be used to stir the puck of cellulose into solution after adding liquid.	

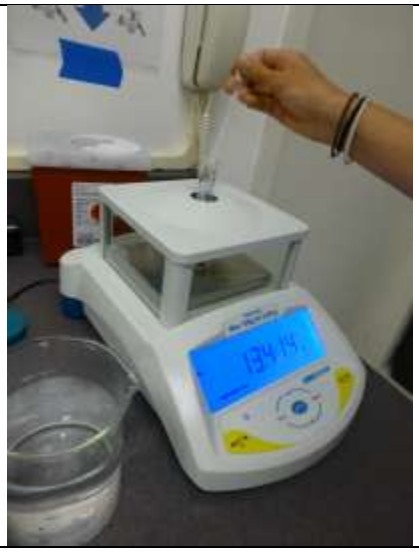
18. Repeat steps 13-17 until cellulose solution is completely divided evenly amongst the vials.

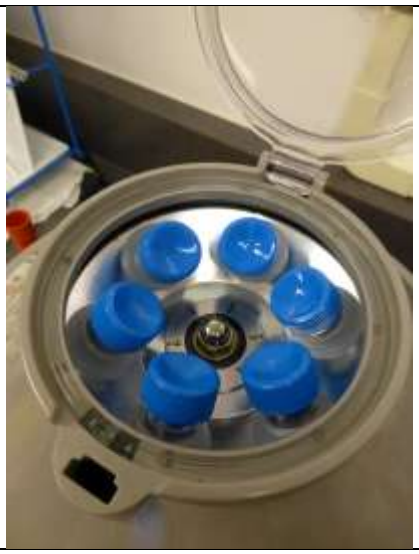
19. Fill each vial to about 14mL with DI water. Again, make sure the weights of vials are equal.	

20. Repeat steps 15-17 until each vial has undergone 4 water washes. Pour off liquid from each vial into the waste beaker after the last water wash.

Solvent Exchange

1. Put on appropriate lab safety equipment. (goggles, gloves, apron, etc...)

2. Using a plastic pipette, fill each vial to about 14mL with solvent. Again, make sure the weights of vials are equal.	

3. Place filled and capped vials into the centrifuge slots. Make sure that the vials are distributed evenly to achieve balanced weight. Close the lid.	

4. Run the centrifuge for 2 minutes at 2500 RPM. Adjust the time and RPM using the (+) and (-) buttons if needed.	

5. Pour off liquid ("supernatant") from vials into waste beaker. A "puck" of cellulose should remain at the bottom of the vial. NOTE: A glass or wooden rod should be used to stir the puck of cellulose into solution after adding liquid.	

6. Repeat steps 3-5 until each vial has undergone 4 solvent washes.

7. Transfer cellulose-solvent solution into clean glass vials. Label each vial using a sharpie to identify date created, solvent used, and number of the vial.	

Appendix B: Nanocrystalline Cellulose Filtration Procedure

Standard Operating Procedure

Nanocrystalline Cellulose Filtration Procedure

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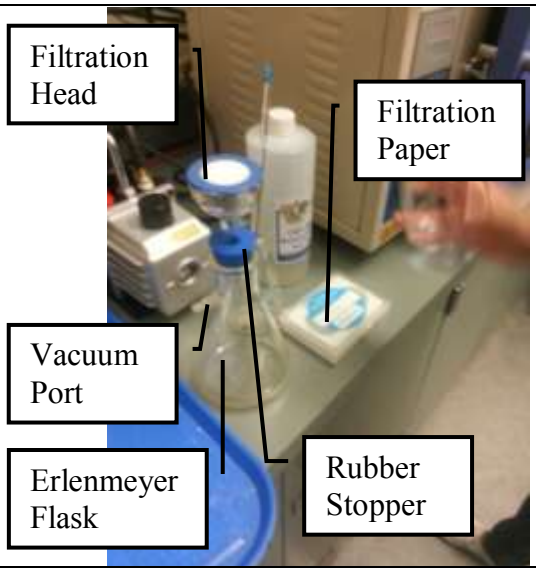
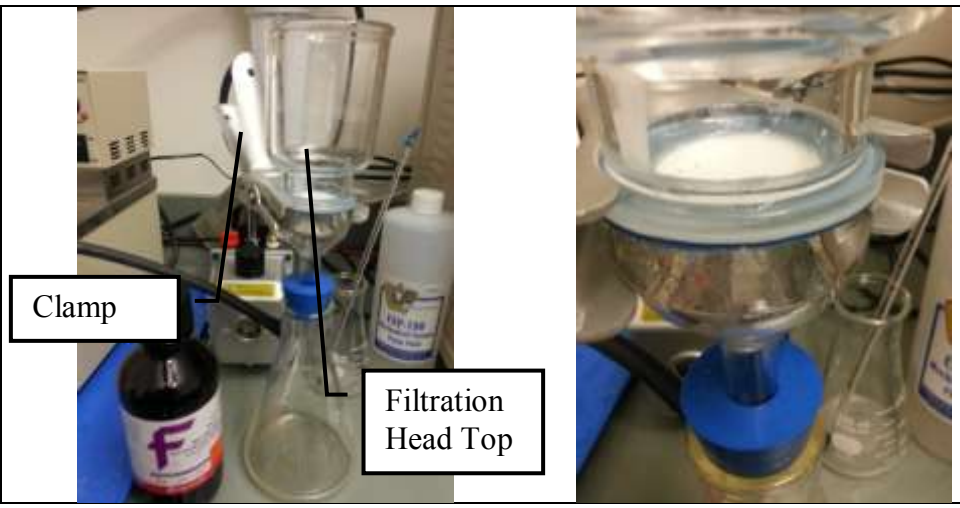
Filtration Set Up

Note: Filtration was performed in Bldg 192 Room 202.

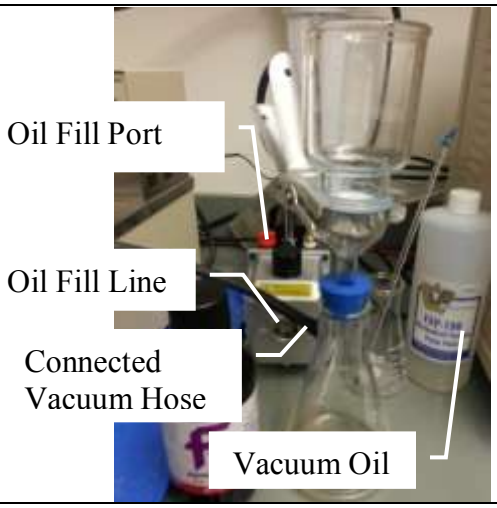
1. Put on gloves and goggles. Obtain cellulose solution and respective solvent.	
2. Obtain erlenmeyer flask with vacuum port, rubber stopper, filtration head, filter paper (0.2 µm). Put rubber stopper in flask. Slide filtration head into rubber stopper. Place one sheet of filtration paper onto top of filtration head.	

3. Secure the filtration head top on the filtration head using the metal clamp.



4. Connect vacuum hose to vacuum port on erlenmeyer flask. NOTE: You may have to disconnect the hose from the vacuum oven in order to do this. Be sure to reconnect the hose when finished.

   NOTE: Make sure the oil in the vacuum is above the minimum oil line before operating vacuum. Fill vacuum with oil if necessary.

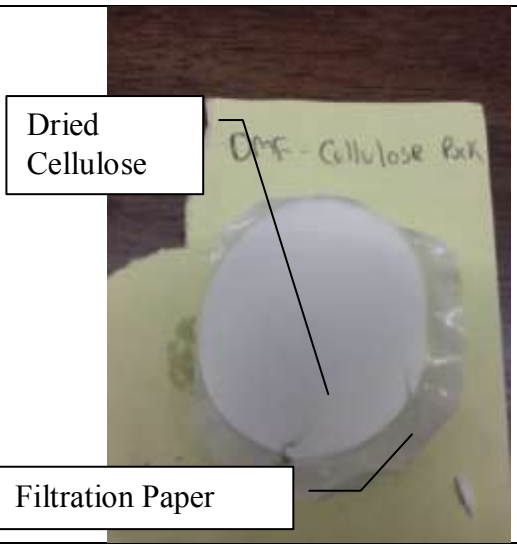


5. Start vacuum.

6. Pour cellulose solution through the filtration paper. Add more additional solvent as necessary.

7. Turn off vacuum.

8. Disassemble filtration apparatus.

9. Place filtration paper and dry cellulose in solid waste container located under the fume hood in Bldg 192 Room 212.	

10. Pour the filtered NCC solution into a clean glass vial. Label the vial with the date and contents using sharpie.	

11. Reconnect vacuum hose to vacuum oven. Clean up lab area and return lab equipment to its appropriate location.

Appendix C: Arabinan and Cellulose Based Nano-Composite Casting Procedure

Standard Operating Procedure

Arabinan and Cellulose Based Nano-Composite Casting Procedure

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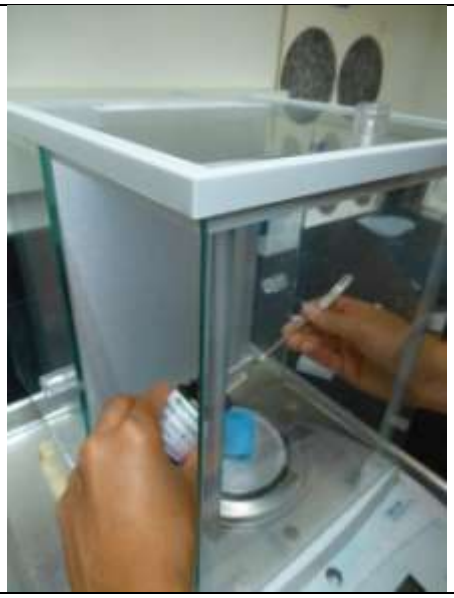
Casting Procedure

Note: Samples were prepared in Bldg 192 Room 212.

*Keep log of all casting conditions and parameters.

Stirring

1. With all of the scale's doors closed, and empty weigh-boat on scale "zero" the scale.	
2. Using a thin metal spoon, scoop out the arabinan onto the weigh-boat until you reach the desired amount. Then set aside.	

3. With an empty 100mL beaker on the scale "zero" the scale.	

4. Add the desired amount of filtered nanocrystalline (NCC) to the 100mL beaker. Cover and set aside.	

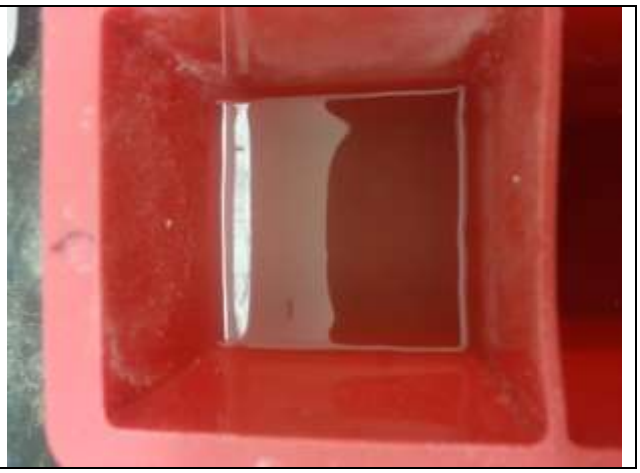
5. With an empty 100mL beaker on the scale "zero" the scale.	

6. Measure out the required amount of solvent to achieve the desired weight% solution. NOTE: this may have to be done in two steps since if scale reaches its max weight capacity. Cover and set aside.	

7. Add arabinan, filtered ncc, solvent, and rounded stir bar to flat bottom reaction vessel. Place cap on reaction vessel. Put reaction vessel on hot plate. Turn stir knob to “4”. Do NOT turn on heat.	

7. Create label for reaction (user’s name/contact info, contents). Allow to stir for 24 hours.

Casting

8. Pour stirred solution into silicone tray.	

Drying

9. Either dry the sample at room temperature under a fume hood or place in vacuum oven. When using the vacuum oven turn power on, set temperature (in °F) and allow to heat. NOTE: The temperature will overshoot by about 10 °F before falling to desired temperature. When using the vacuum pump, ensure the pump has a sufficient amount of oil. Do not leave vacuum pump running for more than 3 hours without checking oil level. Refer to operating manual for vacuum oven and pump for further instructions.	

10. Allow the sample to dry for 24-48 hours.

8. References

[1] Gindl-Altmutter, Wolfgang, and Jozef Keckes. "The Structure and Mechanical Properties of Spines from the Cactus Oputia Ficus-Indica." *BioResources*. N.p., 26 Jan. 2012. Web. 20 May 2013. <http://ojs.cnr.ncsu.edu/index.php/BioRes/article/view/BioRes_07_1_1232_Gindl_K_Structure_Prop_Spines_Cactus/1394>.

[2] Huber, Tim, Jorg Mussig, Shusheng Pang, Simon Bickerton, and Mark P. Staiger. "A Critical Review of All-cellulose Composites." Journal of Materials Science47 (2011): 1171-186. Print.

[3] Varki A, Cummings R, Esko J, Freeze H, StanleyP, Bertozzi C, Hart G, Etzler M (2008). "Essentials of glycobiology". *Essentials of Glycobiology* (Cold Spring Harbor Laboratory Press; 2nd edition). ISBN 0-87969-770-9.

[4] Vignon, M. R., L. Heux, M. E. Malainine, and M. Mahrouz. "Arabinan-cellulose composite in *Opuntia ficus-indica* prickly pear spines." Carbohydrate RESEARCH 339.1 (2004): 123-31. Print.

[5] This information came from the data sheet and information provided with the purchase of the arabinan.

[6] Bondeson, Daniel, Aji Mathew, and Kristiina Oksman. "Optimization of the Isolation of Nanocrystals from Microcrystalline Cellulose by Acid Hydrolysis." *Cellulose* 13 (2006): 171-80. Print.

[7] Malainine, Mohamed E., Alain Dufresne, Daniele Dupeyre, Mostafa Mahrouz, Roger Vuong, and Michel R. Vignon. "Structure and morphology of cladodes and spines of *Opuntia Ficus-indica*. Cellulose extraction and characterization."*Carbohydrate Polymers* 51 (2003): 77-83. *Elsevier*. 2003. Web. 3 June 2013.

[8] Nishiyama, Yoshiharu; Langan, Paul; Chanzy, Henri (2002). "Crystal Structure and Hydrogen-Bonding System in Cellulose Iβ from Synchrotron X-ray and Neutron Fiber Diffraction". *J. Am. Chem. Soc* **124** (31): 9074–82.doi:10.1021/ja0257319. PMID 12149011..

[9] Bai, Wen, James Holbery, and Kaichang Li. "A Technique for Production of Nanocrystalline Cellulose with a Narrow Size Distribution." *Cellulose* (2009): 1-22. 11 Feb. 2009. Web. 1 Oct. 2012.